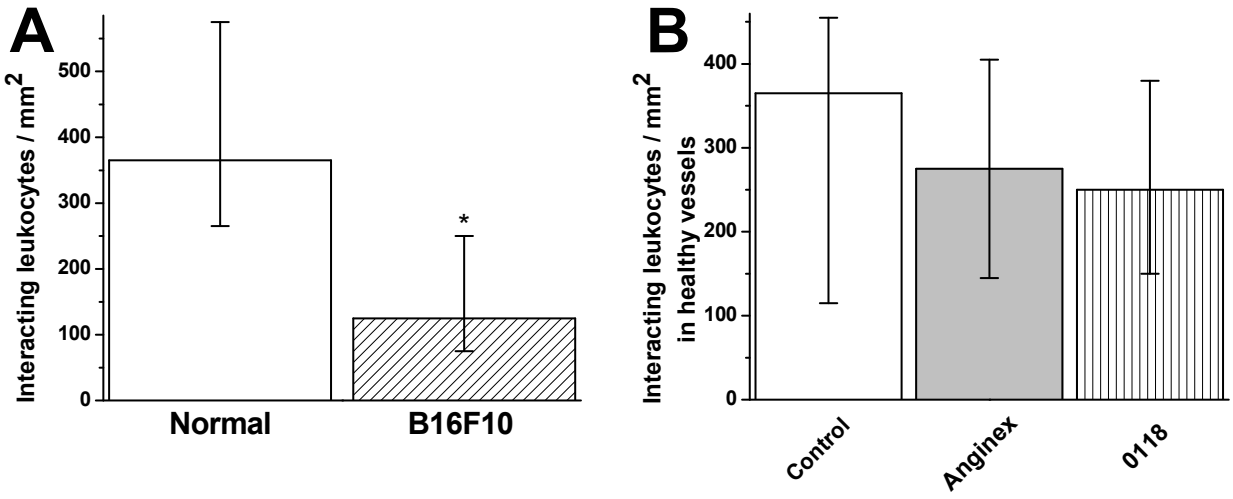
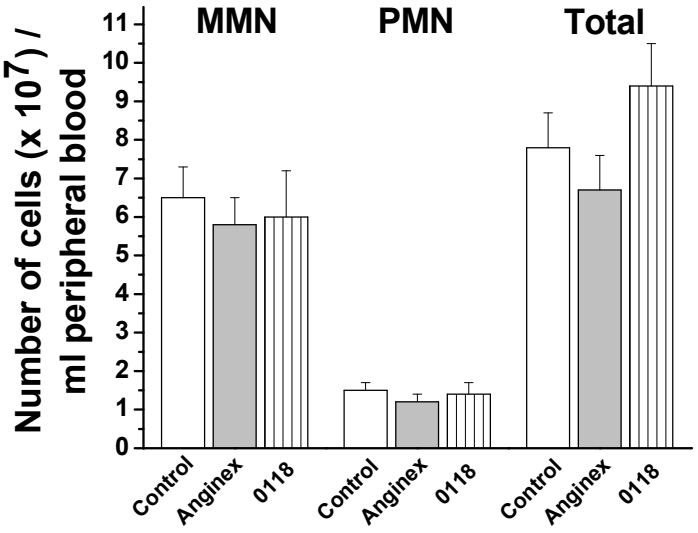
Supplemental Data

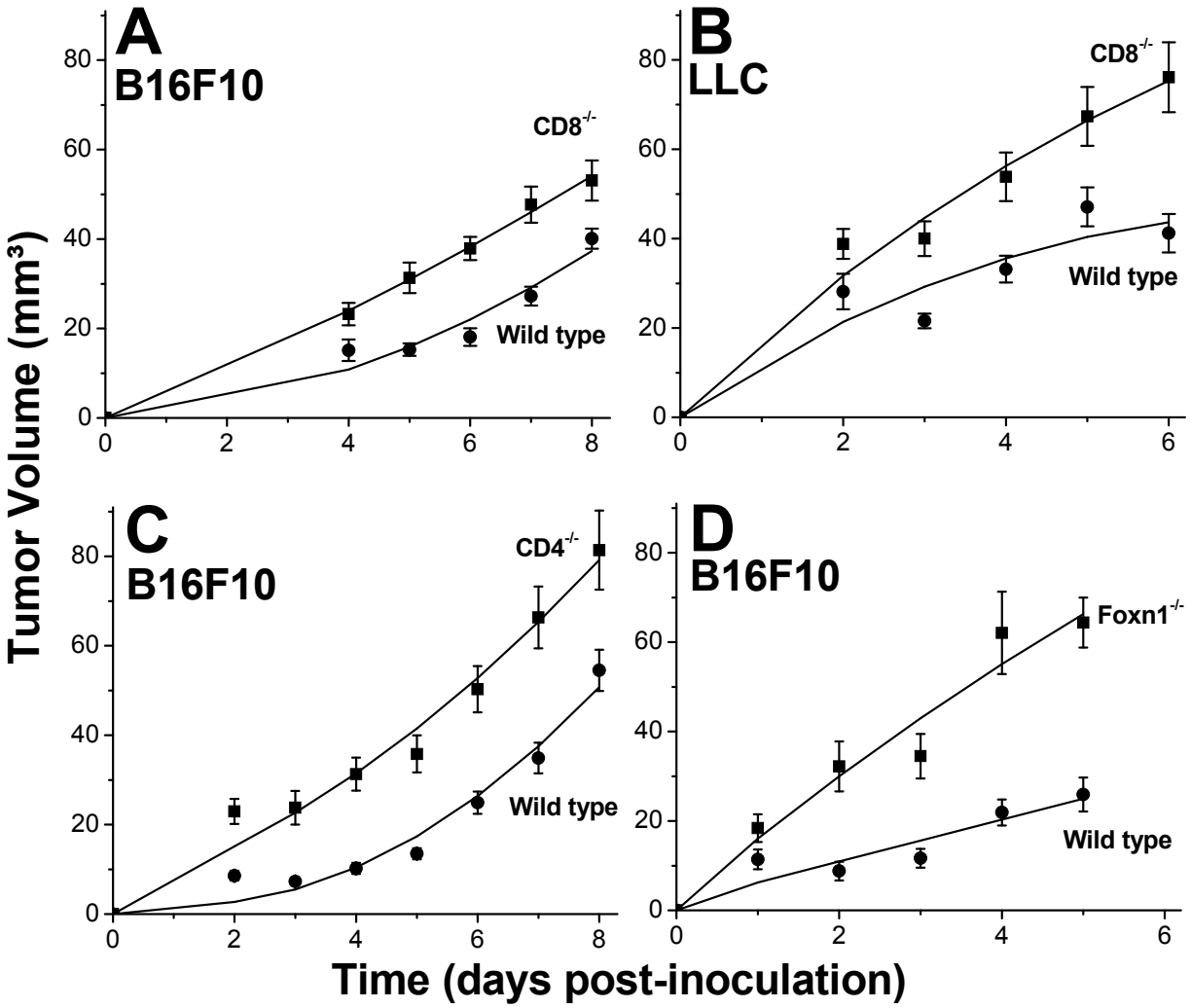
Supplemental data to the manuscript: ‘Enhancement of T-cell mediated anti-tumor response: angiostatic adjuvant to immunotherapy against cancer’ by RPM Dings *et al*.



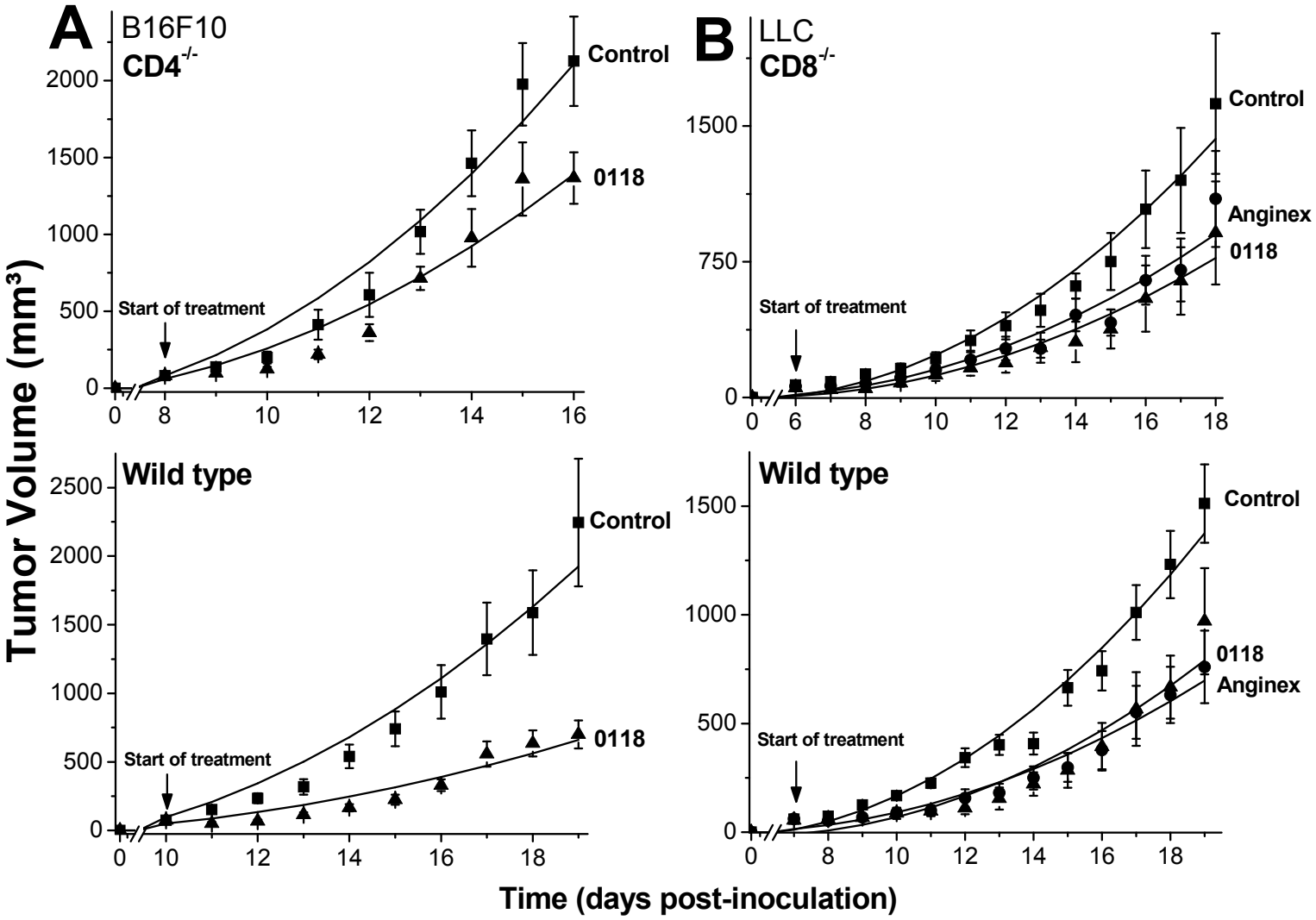
Supplemental Figure S1. Quantifications of images from intravital fluorescence microscopy using rhodamine-labeled leukocytes in healthy tissue or in treated or untreated B16F10 tumors. (**A**) Interacting leukocytes per mm^2 vessel surface in vessels of healthy tissue compared to vessels in B16F10 tumors from non-treated mice. (**B**) Interacting leukocytes per mm^2 vessel surface in healthy vessels.



Supplemental Figure S2. Effect of angiostatic treatment on circulating leukocytes. The number of circulating leukocytes (PMN and MMN) per ml peripheral blood (mean values ± SEM) from angiostatically treated (n=2) or untreated (n=2) animals.

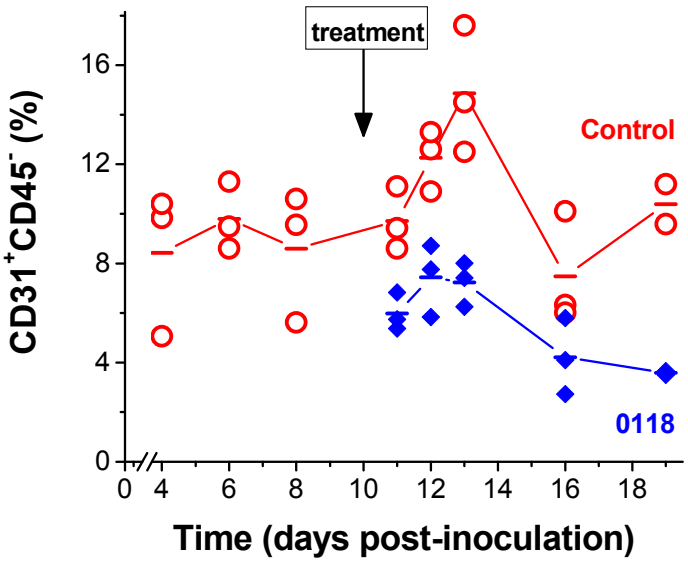


Supplemental Figure S3. Tumor progression in T cell deficient mice. Tumor growth in wild type and T cell null mice is plotted vs. time from inoculation. (**A**) B16F10 tumor growth in wild type (n=101) and $CD8^{-/-}$ (n=51) mice. (**B**) Lewis lung carcinoma (LLC) tumor growth in wild type (n=36) and $CD8^{-/-}$ (n=36) mice. (**C**) B16F10 tumor growth in wild type (n=42) and $CD4^{-/-}$ (n=20) mice. (**D**) B16F10 tumor growth in wild type (n=20) and $Foxn1^{-/-}$ (n=24) mice. The wild type groups had a significantly lower least squares means of ln(tumor volume +1) compared to $CD8^{-/-}$ B16F10 (P=0.02), $CD8^{-/-}$ LLC (P<0.0001), $CD4^{-/-}$ B16F10 (P=0.004) and $Foxn^{-/-}$ B16F10 (P=0.008), as determined by ANOVA. Overall, tumor growth in wild type mice was delayed by about 2 days compared to $CD8^{-/-}$ or $CD4^{-/-}$ mice (up to 45% tumor growth inhibition), and by about 3 days compared to Foxn-$1^{-/-}$ mice, or about 60% tumor growth inhibition on day 5. The means (± SEM) of 2 or 3 independent studies are shown with polynomial curve fitting. The mice were with sex- and age-matched with wild type littermates and inoculated at random. Tumor size measurements were done blinded.



Supplemental Figure S4. B16F10 and LLC tumor growth. Growth curves for B16F10 and LLC tumors in wild type mice and T cell deficient mice are shown with and without angiostatic treatment. (**A**) B16F10 tumor progression was monitored in $CD4^{-/-}$ or wild type mice treated with 0118 (10 mg/kg IP BID for 8 or 9 days, respectively) compared to untreated matched control mice (n=10-20 per group).

(**B**) LLC tumor growth was monitored in $CD8^{-/-}$ or wild type mice treated with anginex or 0118 (both 10 mg/kg IP BID for 12 days) compared to untreated matched control mice (n=12-27 per group). Data are shown as the mean ± SEM of 2 or 3 independent studies with polynomial trendline curve fitting. Wild type and null mice were age, size, and sex matched in each study, and were simultaneously and randomly s.c. injected with 2×10^5 B16F10 (**A**) or 1×10^6 LLC (**B**) cells on the right hind flank.



Supplemental Figure S5. Reduction in tumor endothelial cells by 0118 treatment.Decline of tumor endothelial cells by 0118 treatment during B16F10 tumor growth. Treatment with 0118 (10 mg/kg IP BID) was initiated on day 10 post inoculation with cultured tumor cells. Mean fluorescence intensities (MFI) are shown for tumor-derived cell suspensions from individual mice, and lines connect the mean values determined on each day examined.

Supplemental Table S1.

Fluid dynamic parameters in tumor vessels of treated and non-treated mice. [a]

	Control	Anginex	0118
n_m [b]	6	7	8
n_v [b]	37	31	37
Diameter (μM)	35 (25-40)	35 (25-40)	30 (25-40)
Centerline velocity (mm/s)	0.8 (0.2-0.9)	0.9 (0.7-1.4)	1.1 (0.8-1.4)**
U (s^{-1}) [b]	13.2 (10.16-18.95)	14.3 (11.72-23.44)	20.1 (13.6-29.5)**
Q (mm^3/s) [b]	$4.1x10^4$ ($1.8-8.1x10^4$)	$5.9x10^4$ ($3.3-23.4x10^4$)	$5x10^4$ ($3-8.4x10^4$)

[a] Data are presented as median values with interquartile ranges. Statistical significance was assessed in comparison to values in untreated mice (**$p<0.005$)
[b] n_m, number of mice; n_v, number of vessels; *U* reduced velocity; *Q* flow

Supplemental Table S2.

Sequences of primers used in quantitative real-time RT-PCR.

Species	*Forward Target*	*Accession Number*	*Reverse primer 5'-3'*	*Forward primer 5'-3'*
h	β-Actin	X00351	CCTGTGTGGACTTGGGAGAG	CATTCCAAATATGAGATGCATT
m	β-Actin	X03672	GGAGGAAGAGGATGCGGCA	GAAGCTGTGCATGTTGCTCTA
h	ICAM-1	X06990	TAGACACTTGAGCTCGGGCA	GGCCGGCCAGCTTATACAC
m	ICAM-1	NM_010493	CGTCTTGCAGGTCATCTTAGGAG	GTGGCGGGAAAGTTCCTG
h	VCAM-1	X53051	ACTCCTCACCTTCCCGCTC	TCAGATTGGAGACTCAGTCATGT
m	VCAM-1	NM_011693	CATTCCTTACCACCCCATTG	AGTTGGGGATTCGGTTGTTC
h	E-selectin	NM_000450	TAAAGCCCTCATTGCATTGA	CCCGAAGGGTTTGGTGAG

Primer sequences are shown as large caps (5′–3′); h = human; m = mouse